

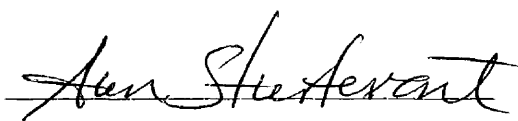
**Molecular Genetics of Michigan's Only  
Population of the Six-Lined Racerunner  
Lizard, *Aspidoscelis sexlineata*.**

By

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in partial fulfillment of the requirements for the degree of  
Master of Science in Biology, University of Michigan-Flint

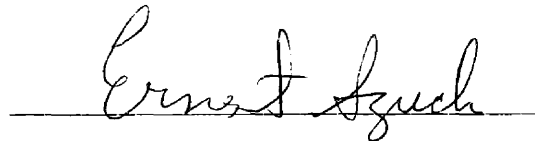
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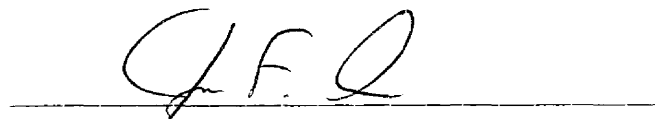
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June 2008  
Flint, Michigan

## **Abstract**

Molecular genetics information about Michigan's only population of six-lined racerunner, *Aspidoscelis sexlineata*, was obtained by sequencing a mitochondrial gene, ND2. The ND2 sequence of the Michigan population was compared to ND2 sequences from geographically near and distant populations. A 50% majority rule consensus tree and a bootstrap consensus tree were generated using PAUP\*(4.0). It shows that the Michigan six-lined racerunner population is most closely related to other Midwestern populations (Indiana Dunes, Nebraska and Arkansas), and is more distantly related to Southern (Florida) and Western (Texas) populations.

The close proximity of the Indiana Dunes Region to Michigan, and the pre-settlement maps that show an extension of the savanna habitat from Indiana to southern Michigan, gave rise to the hypothesis that the Michigan six-lined racerunner population may be endemic. According to this theory the six-lined racerunner lizard dispersed from a neighboring Midwestern population. In Michigan, the population became isolated by road building and agricultural fields. Our molecular data support this scenario, since the Michigan ND2 sequences

are very similar to ND2 sequences from Indiana and Nebraska. The molecular data appear to contradict the alternate theory, which says the six-lined racerunner lizard was introduced from the Southern or Western US, presumably by human intervention. If this were the case one would expect the Michigan ND2 sequences to be most similar to sequences from the Southern and Western states; however, the molecular data shows the ND2 sequences from Florida and Texas have numerous differences compared to the Michigan and other Midwestern ND2 sequences.

## **Acknowledgements**

I thank Dr Ann Sturtevant for being a great advisor. I thank Ernie and Annie Szuch, Joseph Sucic, Teresa Yoder, Ashley Cornett and Nickole Hatley for their help and advice.

I thank my family and friends for their love and support.

I thank Fulbright Foreign Student Program for the financial support it provided during my time at UM-Flint.

Financial support for this project was provided by the College of Arts and Sciences at University of Michigan-Flint.

Also, I thank Larry Atherton for helping to order the lab supplies and kits.

Finally, I thank those who provided me with samples:

Teresa Yoder, Biology department, University of Michigan-Flint, Flint, MI.

Florida Fish and Wildlife Conservation Commission, Wildlife Research Laboratory, Tallahassee, FL.

Dr Stan Trauth, Department of Biological Sciences, Arkansas State University, State University, AR.

Dr. John B. Iverson, Department of Biology, Earlham College, Richmond, IN.

The Division of Amphibians and Reptiles, The Field Museum,  
Chicago, IL

Dr Michael Forstner, Department of Biology, Texas State  
University, San Marcos, TX.

# Table of Contents

Abstract .....	ii
Acknowledgements .....	iv
List of Figures .....	vii
List of Tables .....	viii
The Project Goal .....	1
Background Information.....	1
Materials and Methods .....	12
Results .....	22
Discussion .....	39
References .....	50

## **List of Figures:**

Figure 1: <i>Aspidoscelis sexlineata</i> Two Subspecies.....	2
Figure 2: Distribution Map of the Racerunner Lizard, <i>Aspidoscelis sexlineata</i> .....	3
Figure 3: Murphy Lake State Game Area Map.....	7
Figure 4: Map of Animal Mitochondrial DNA .....	10
Figure 5: Primers Used for PCR and DNA Sequencing of the ND2 and tRNA <sup>trp</sup> Region .....	17
Figure 6: Alignment of ND2 and tRNA <sup>trp</sup> Sequences of <i>Aspidoscelis sexlineata sexlineata</i> (A.s.s), <i>Aspidoscelis sexlineata viridis</i> (A.s.v) and <i>Ameiva chrysolema</i> (Ameiva c.) .....	23
Figure 7: 50% Majority-rule Consensus Tree of 20 <i>Aspidoscelis sexlineata</i> Samples .....	35
Figure 8: Bootstrap Consensus Tree from 20 <i>Aspidoscelis sexlineata</i> Samples.....	38
Figure 9: Map of Pre-settlement Vegetation of Michigan, circa 1800 .....	43
Figure 10: Map of Pre-settlement Vegetation of Indiana, circa 1820.....	44
Figure 11: Map of Current Vegetation in Michigan, circa 2004.....	46

## **List of Tables**

Table 1: Lizard Tissue Samples and Their Localities .....	12
Table 2: Primer Pairs Used in the Amplification of the Mitochondrial ND2 and tRNA <sup>trp</sup> Region .....	18



## **The Project Goal**

The main goal of this study is to provide molecular genetics information about Michigan's only population of Six-lined Racerunner, *Aspidoscelis sexlineata*. One mitochondrial gene, NADH Dehydrogenase subunit 2 (ND2), was sequenced and compared among populations of Six-lined Racerunner from different localities in the United States. This study will provide molecular evidence that will be used to answer questions about the origin of Michigan's Six-lined Racerunner population.

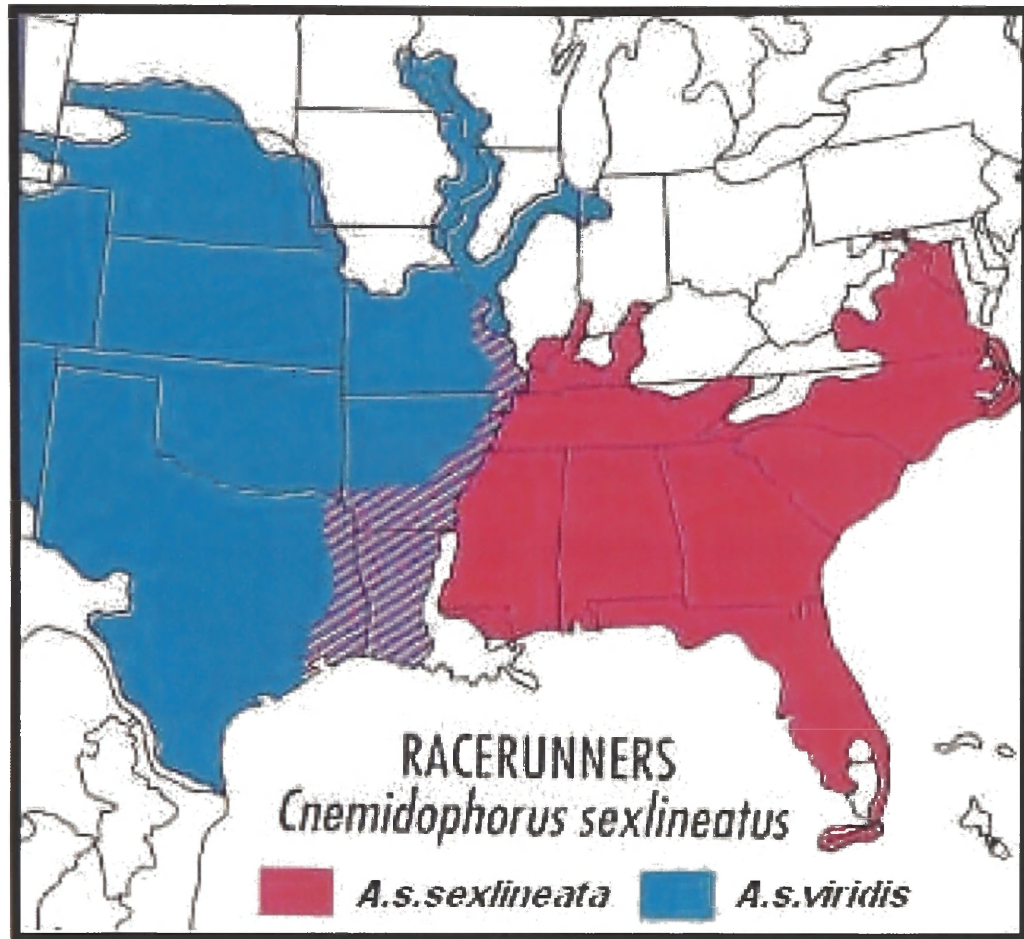
## **Background Information**

Six-lined racerunners, *Aspidoscelis sexlineata*, formerly known as *Cnemidophorus sexlineatus*, are whiptail lizards from the Teiid family. Two subspecies have been described, *Aspidoscelis sexlineata sexlineata* and *Aspidoscelis sexlineata viridis*. This burrowing lizard is light brown and has light yellow stripes along its body. The subspecies *A. s. sexlineata* has a dark green-brown or black dorsal color, and six light yellow stripes extending along the body. It is found from Maryland in the north to Florida in the south and west to Montana and eastern Texas. The subspecies *A. s. viridis* has a light green dorsal color

and seven light stripes. Males have a pale blue ventral surface anteriorly. *A.s.viridis* is found from southern South Dakota and eastern Wyoming, south to southern Texas and northeast through Montana to the extreme northwestern part of Indiana and north along the Mississippi River Valley into southeastern Minnesota. The range of the two subspecies overlaps west of the Mississippi River, from St. Louis to the Gulf Coast of western Louisiana and eastern Texas (Conant and Collins, 1998) (Figure 1; Figure 2).



**Figure 1: The Two Subspecies of *Aspidoscelis sexlineata*.** *Aspidoscelis sexlineata sexlineata* (left) has six light yellow lines along the body. *Aspidoscelis sexlineata viridis* (right) has seven light yellow strips and a green dorsum. Males have a light blue ventral surface anteriorly.



**Figure 2: Distribution Map of the Racerunner Lizard, *Aspidozelis sexlineata*** (Conant and Collins, 1998). *Aspidozelis sexlineata viridis* is found in the Midwest and central and western areas. *Aspidozelis sexlineata sexlineata* is primarily found in the south-southeast. The range of the two subspecies overlaps in Louisiana, east Texas and parts of Arkansas and Missouri near the Mississippi River.

An isolated and unique *Aspidoscelis sexlineata* population was discovered in the late 1980's in the state of Michigan (Harding and Holman, 1990). It is found along a south facing hillside adjacent to a busy road in the Murphy Lake State Game Area (MLSGA). The area is composed of sandy soils with grassy vegetation. A few trees are scattered around the habitat (Yoder, 2007). The nearest *Aspidoscelis sexlineata* population is found in the Indiana Dunes Region, over 322 km away (Conant and Collins, 1998; Harding, 1997). Morphological and population characteristics of *Aspidoscelis sexlineata* in Michigan have been recently described (Yoder, 2007). Based on morphological features, the population appears to be the prairie subspecies, *Aspidoscelis sexlineata viridis*. Population size estimates are well over 450 individuals for the entire area occupied by the lizards. Hatchling survivorship varies widely by year. The active period for this population is longer than is listed in most references but seems to coincide with the other northern populations (Harding, 1997; Kapfer and Pauers, 2006). All vegetation types within the area occupied were used by the lizards; however, bare/sparse vegetation and thick vegetation were used more frequently than any other

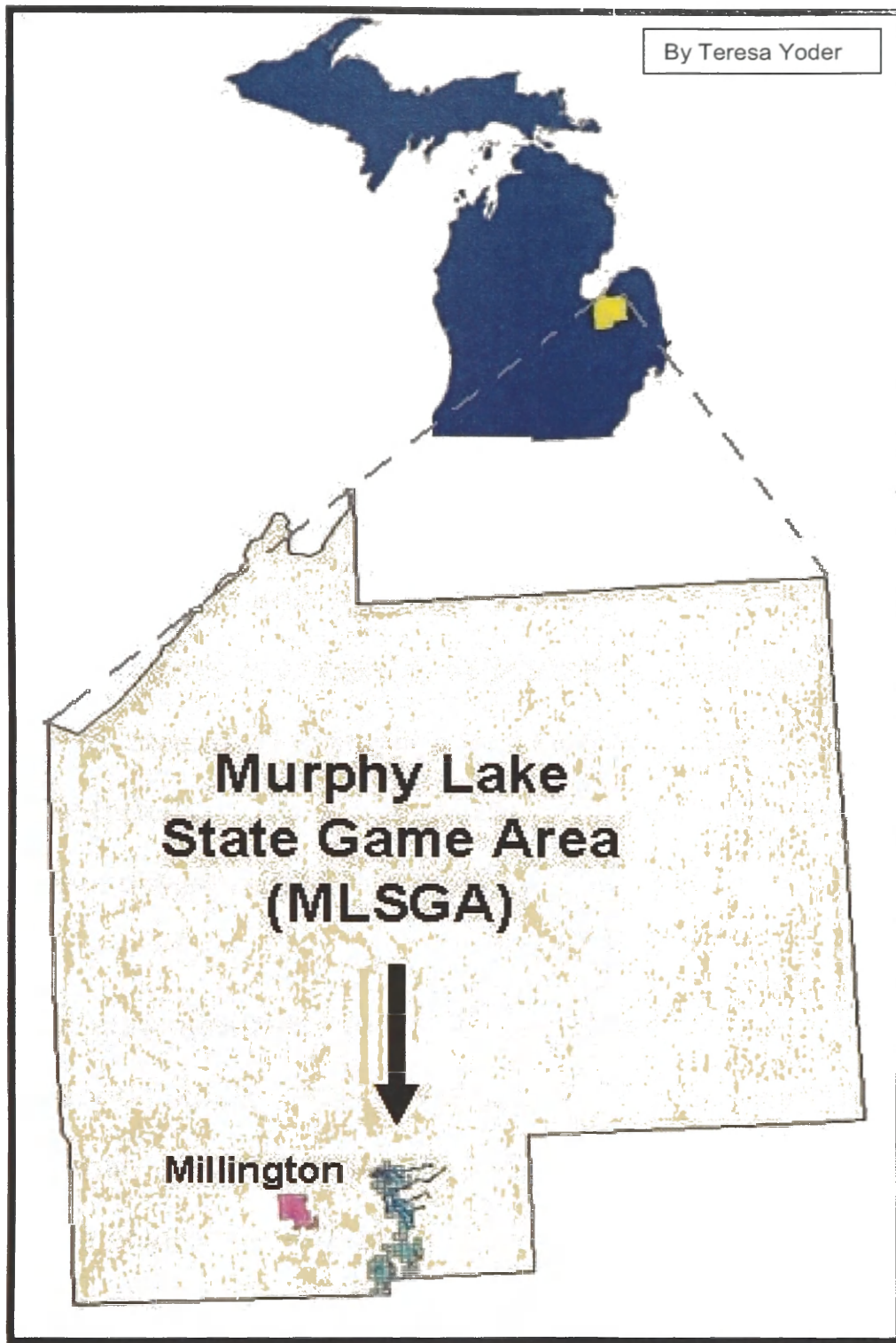
vegetation cover type. Potential competitors or predators were rarely found in the area (Yoder, 2007).

*Aspedoscelis sexlineata* lives in relatively dry regions on sandy or other loose soil, in short grass, sparse woods, or areas with scattered, subxerophytic vegetation (McFarlane, 1999). Racerunners may wait until May to begin their seasonal activity period. Racerunners bask atop rocks or logs and actively forage for their food. Prairie racerunners have been clocked at speeds of 18 miles per hour (Vogt, 1981). They eat small invertebrates such as crickets, grasshoppers, beetles, spiders and caterpillars, which they chase down and eat. They usually begin hibernating in September (McFarlane, 1999).

The Great Lakes area was reformed after the end of the last glacial period 10,000 years ago. It radically altered the geography of North America north of the Ohio River. The grooves and end moraines left by these glaciers can be easily observed. During the latest glacial period, many organisms migrated south and continued to survive in warmer areas. As the ice sheets retreated, species slowly dispersed back north and inhabited the newly formed areas.

Murphy Lake State Game Area (MLSGA) is located three miles east of the village of Millington, in southern Tuscola County, Michigan (Figure 3). It is about 10.7 km<sup>2</sup> of habitat islands in the middle of an agricultural landscape. Steep end-moraine ridges, and pitted outwash deposits make up the game area (MLSGA Strategic Plan 2004). MLSGA has unique fauna compared to other game areas in the region. It is home to the only known populations of the Northern Dusky Salamander *Desmognathus fuscus* (Yoder 2007) and the Six-lined Racerunner lizard *Aspedoscelis sexlineata* (Harding and Hollman 1990) in the state of Michigan. Northern Dusky Salamanders dwell along the streams of the hemlock forests and less than a mile away, the racerunner lizards live on a grassland habitat along the side of a road.





**Figure 3: Murphy Lake State Game Area Map.** Murphy Lake State Game Area, Tuscola County, MI has unique habitats interspersed in a forested area. Consequently, unique fauna such as *Aspidoscelis sexlineata* and *Desmognathus fuscus* are found in the area.

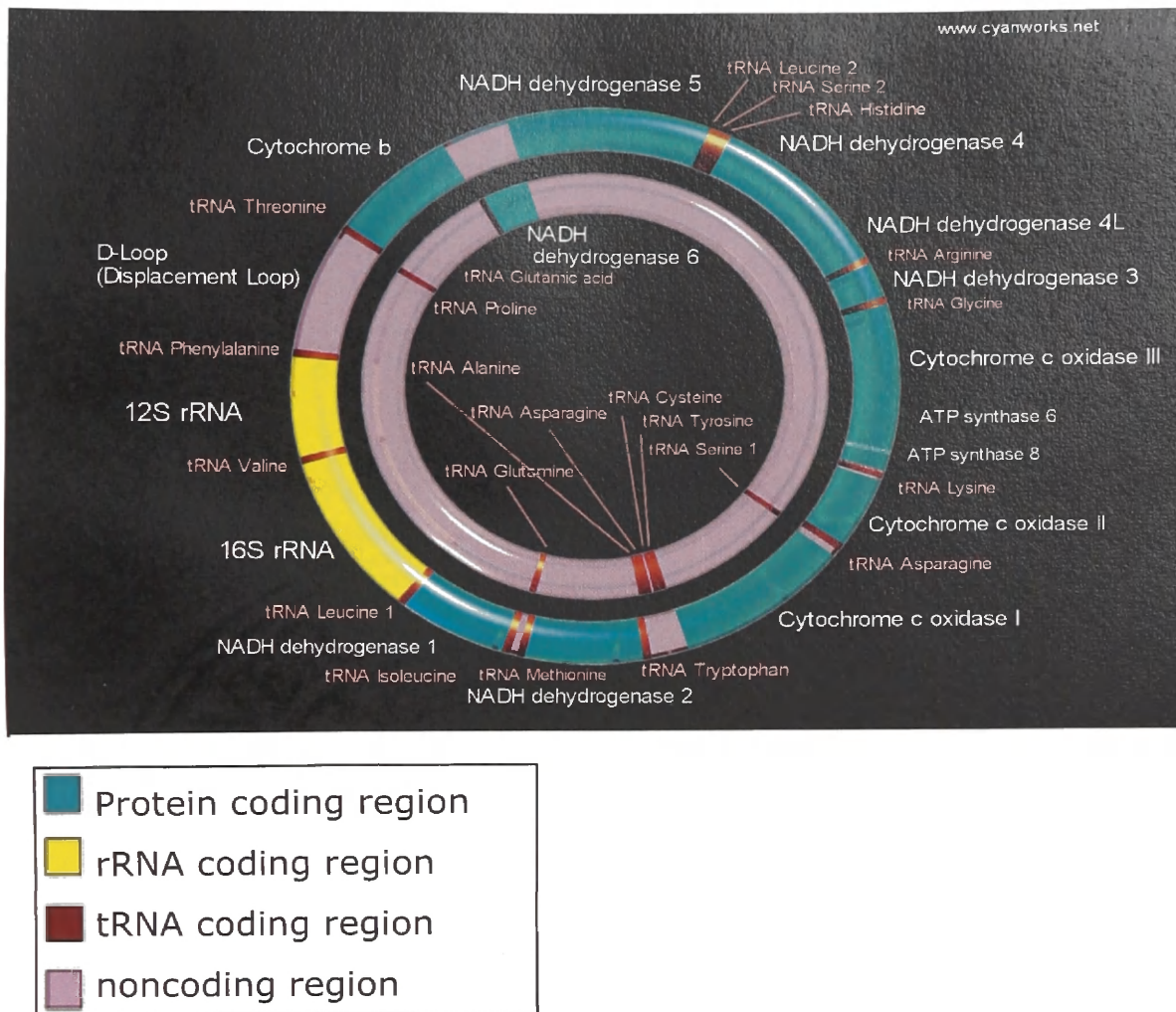
Being unique and isolated, the presence of *A. sexlineata* population Michigan provokes many questions. How did these lizards come here after the glacial retreat? Did they naturally disperse from a close population, perhaps the one in Indiana dunes? Or have humans introduced them to Murphy Lake State Game Area from the southern or western states?

Molecular phylogenetics is the use of nucleotide sequence of DNA or the amino acid sequence of proteins to gain information on an organism's evolutionary relationships. Molecular data can be use to construct phylogenetic trees. Phylogenetic trees are representations of the simplest path through which related taxa may have descended from a common ancestor.

Nuclear and mitochondrial DNA have been used in molecular phylogenetic studies. Yet, mitochondrial DNA evolves five times faster than nuclear DNA (Brown, 1979). It is the highly oxidative environment in the mitochondrion that makes the rate of mutation higher than that in the nucleus. The mitochondrial genome is a very compact genome that contains very few non-coding regions of DNA. The small size of the vertebrate mitochondrial genome ranges from 16-19 Kb. One DNA strand,



the L strand, encodes only one protein-coding gene (NADH dehydrogenase 6) and eight tRNA genes. The remaining 12 protein-coding genes, two rRNA and eight tRNA genes are all located on the H strand. A major noncoding region, the control region is also located on the H strand. (Boore 1999; Pereira 2000; Fairbanks, 1999). The mitochondrial genome is maternally inherited which renders genetic recombination extremely rare (Figure 4).



**Figure 4: Map of Animal Mitochondrial DNA.** NADH dehydrogenase 2 and tRNA<sup>trp</sup> are located on the heavy strand of the Mitochondrial DNA.

NADH dehydrogenase 2 (ND2), a mitochondrial gene, is the most widely used gene for phylogenetic studies in lizards (Macey, 1997; Macey, 1999; Sorenson, 2003). It is the third most variable gene after ATPase 8 and NADH dehydrogenase 6 (ND6). ATPase 8 is very short (~165-168 bp) and therefore provides relatively little information. ND6 is also relatively short (~519-522 bp) and difficult to amplify and sequence, given its

unusual base composition and location near the control region (Sorenson, 2003). NADH dehydrogenase 3 (ND3) and NADH dehydrogenase 4 (ND4) of the same Michigan racerunner population at MLSGA were sequenced and compared to other populations, yet no conclusive results were obtained (Razzano, 1999). ND 3 is very short (~380 bp) and ND 4 (~900 bp) apparently did not have enough differences to resolve sequences from the same population or subspecies into separate taxa in a phylogenetic tree. For these reasons, I chose to use ND2 for my sequences.

Although we cannot absolutely determine the origin of the Michigan population, some scenarios are more likely to have occurred. If the six-lined racerunner lizard was introduced from the Southern or Western US, presumably by human intervention, one would expect the Michigan DNA sequences to be most similar to sequences from the Southern and/or Western states. If the six-lined racerunner lizard dispersed from a neighboring Midwestern population, the Michigan DNA sequences should be very similar to sequences from the Midwest.

## **Materials and Methods**

### **Sample collection**

Michigan samples were collected by Teresa Yoder, Biology Department, University of Michigan-Flint, Flint, MI. Florida samples were obtained from The Wildlife Research Laboratory, Florida Fish and Wildlife Conservation Commission, Tallahassee, FL. Arkansas samples were obtained from Dr Stan Trauth, Department of Biological Sciences, Arkansas State University, State University, AR. Nebraska samples were obtained from Dr. John B. Iverson, Department of Biology, Earlham College, Richmond, Indiana. Indiana samples belong to The Division of Amphibians and Reptiles, The Field Museum, Chicago, IL. Texas samples belong to Dr Michael Forstner, Department of Biology, Texas State University, San Marcos, TX. *Ameiva chrysolaema* sequence was obtained from Genbank (accession number: AY561703). Samples are summarized in Table 1.

**Table 1: Lizard Tissue Samples and Their Localities.**

Lizard Species and subspecies	Animal Number	Animal code	Tissue Type	Location
<i>Aspidoscelis sexlineata viridis</i>	MICH3	A.s.v. MI1	Blood on FTA paper	Tuscola county, MI
<i>Aspidoscelis sexlineata viridis</i>	MICH4	A.s.v. MI2	Blood on FTA paper	Tuscola county, MI
<i>Aspidoscelis sexlineata viridis</i>	MICH5	A.s.v. MI3	Blood on FTA paper	Tuscola county, MI
<i>Aspidoscelis sexlineata viridis</i>	MICH7	A.s.v. MI4	Blood on FTA paper	Tuscola county, MI
<i>Aspidoscelis sexlineata viridis</i>	MICH8	A.s.v. MI5	Blood on FTA paper	Tuscola county, MI
<i>Aspidoscelis sexlineata viridis</i>	MICH9	A.s.v. MI6	Blood on FTA paper	Tuscola county, MI
<i>Aspidoscelis sexlineata viridis</i>	MICH22	A.s.v. M7	Blood on FTA paper	Tuscola county, MI
<i>Aspidoscelis sexlineata viridis</i>	INDU27	A.s.v. IN1	Tail	Indiana dunes, Porter county, IN
<i>Aspidoscelis sexlineata viridis</i>	INDU18	A.s.v. IN2	Tail	Indiana dunes, Porter county, IN
<i>Aspidoscelis sexlineata viridis</i>	ASUMZ30322	A.s.v. AR1	Blood on FTA paper	Greene county, AR
<i>Aspidoscelis sexlineata viridis</i>	ASUMZ30324	A.s.v. AR2	Blood on FTA paper	Greene county, AR
<i>Aspidoscelis sexlineata viridis</i>	ASUMZ30323	A.s.v. AR3	Blood on FTA paper	Greene county, AR
<i>Aspidoscelis sexlineata viridis</i>	Sample 1	A.s.v. NB1	Tail	Garden county, NE
<i>Aspidoscelis sexlineata viridis</i>	Sample 2	A.s.v. NB2	Tail	Garden county, NE
<i>Aspidoscelis sexlineata sexlineata</i>	Sample 10	A.s.s. FL1	Blood on FTA paper	Alachua county, FL
<i>Aspidoscelis sexlineata sexlineata</i>	Sample 8	A.s.s. FL2	Blood on FTA paper	Alachua county, FL
<i>Aspidoscelis sexlineata sexlineata</i>	Sample 11	A.s.s. FL3	Blood on FTA paper	Alachua county, FL
<i>Aspidoscelis sexlineata viridis</i>	MF_ID 8616	A.s.v. TX 1	Toe clip	Bastrop county, TX
<i>Aspidoscelis sexlineata viridis</i>	MF_ID 10224	A.s.v. TX 2	Toe clip	Bastrop county, TX
<i>Aspidoscelis sexlineata viridis</i>	MF_ID 16986	A.s.v. TX 3	Toe clip	Bastrop county, TX
<i>Ameiva chrysolaeama</i>	-	Amaeva c.	-	Isla Saona, Mano Juan, Hispaniola

Species and subspecies, tissue type and location of samples are shown.

## **DNA Extraction**

### **Blood Samples**

DNA was extracted from the blood samples spotted onto Whatman FTA paper using the procedure by Smith and Burgoyne (2004) with the following modifications. A sterile Harris Micro-Punch was used to cut a 1.2 mm disc from the spotted blood on Whatman FTA paper. When the blood spots were faint more than one disc was obtained. The discs were put into 1.5 ml tubes. One ml of 100 mM Tris-HCl pH 8.0, 1% SDS was added to each disc and agitated for 30 minutes to lyse the cells on the paper. The buffer was discarded and 500µl of CTAB extraction buffer (20 mM EDTA, 1.4 M NaCl, 100 mM Tris-HCl pH 8.0, 2% CTAB, 1% PVP, 0.2% βME) was added to discs. The solution was adjusted to a final concentration of 1% sarcosyl, and the discs were incubated for 10 minutes at room temperature to lyse the blood cells. The buffer was discarded and 500 µl ddH<sub>2</sub>O was added to the discs. The discs were agitated for 10 minutes then the ddH<sub>2</sub>O was discarded. This ddH<sub>2</sub>O wash was repeated a total of three times. Five hundred µl 95% ethanol was added to the discs. The discs were agitated in ethanol for 10 minutes then the ethanol was discarded. The

discs were transferred to fresh 1.5ml tubes with 50µl ddH<sub>2</sub>O. Tubes were incubated for 15 minutes at 90°C to elute the DNA. Discs and the DNA extract were used for PCR.

### **Tissue Samples:**

A 5-8 mm diameter piece of tail, toe or liver tissue was put into a 1.5ml tube and a pinch of sterile sand was added to assist grinding. A micro-pestle was used to grind the tissue. Four hundred µl of CTAB extraction buffer (20 mM EDTA, 1.4 M NaCl, 100 mM Tris-HCl pH 8.0, 2% CTAB, 1% PVP, 0.2% βME) was added while grinding and mixing, 200 µl at a time. Sarcosyl was added to a final concentration of 1% and the sample was incubated at 60°C for 30 minutes.

The sample was cooled to room temperature and extracted twice with an equal volume of chloroform-isoamyl alcohol (25:1) to remove scales, bone pieces and unhomogenized tissue. The upper aqueous layer was transferred to a clean 1.5 ml tube. An equal volume of 5 M NaCl was added and mixed by inversions. Two thirds of a volume of isopropanol was added and the tube was incubated on ice for at least 15 minutes or at -20°C overnight to precipitate the DNA. The tube was

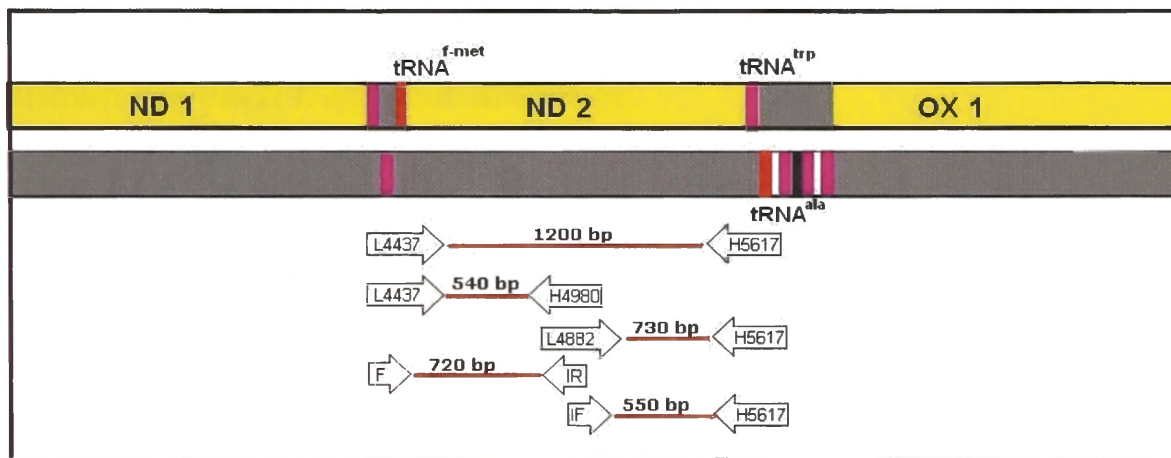
centrifuged at 13,000 x g for 15 minutes. The supernatant was discarded and the pellet was resuspended in 300 µl of 75% ethanol. The tube was centrifuged at 13,000 x g for 5 minutes. The supernatant was discarded. The pellet was air-dried for 5 minutes, then resuspended in 100 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). To digest RNA, 0.1 mg of RNase A was added and the sample was incubated at 37°C for 15 minutes. Two volumes of ddH<sub>2</sub>O, 30 µl of 3 M Na acetate, and 850 µl ethanol were added. The tube was incubated on ice for 15 minutes then centrifuged at 13,000 x g for 15 minutes. The supernatant was discarded and the pelleted DNA was resuspended in 50 µl ddH<sub>2</sub>O.

### **Polymerase Chain Reaction for ND2 and tRNA<sup>trp</sup>**

The PCR reaction mix contained 5-10 µl DNA or one FTA disc. Primer stocks were 10 mM. The PCR mix for one reaction consisted of GoTaq Flexi Green Buffer, 2 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.2 µM forward primer, 0.2 µM reverse primer and 0.02 units Go Taq Flex DNA Polymerase (Promega, Madison , WI). The Mitochondrial ND2 and tRNA<sup>trp</sup> region was either amplified as single fragment of 1200 bp or as two fragments of 730 bp



and 540 bp, or 720 bp and 550 bp that have an overlap of about 100 bp (Figure 5). The smaller two pieces were easier to sequence directly after PCR reaction. The annealing temperature for PCR was optimized for each primer pair.



**Figure 5: Primers Used for PCR and DNA Sequencing of the ND2 and tRNA<sup>trp</sup> Region.** Primers L4437 and H5617 were used to amplify the single 1200bp fragment. Primers L4882 and H5617 yielded the 730bp fragment. Primers L4437 and H4980 yielded the 540bp fragment. Primers IF and H5617 yielded the 550bp fragment. Primers F and IR yielded the 720bp fragment. Primers L4437, H5617 and F were used for sequencing.

**Table 2: Primer Pairs Used in the Amplification of the Mitochondrial ND2 and tRNA<sup>trp</sup> Region.**

<b>Fragment Length bp</b>	<b>Forward Primer</b>	<b>Reverse Primer</b>	<b>Annealing Temperature</b>
1200	L4437	H5617	60°C
730	L4882	H5617	45°C
540	L4437	H4980	50°C
550	I F	H5617	50°C
720	F	I R	50°C

Fragment length, primer pairs and annealing temperature are shown for each fragment amplified.

The following PCR conditions were used: initial denaturation at 94°C for 5:00 minutes, followed by 50 PCR cycles and a final extension of 70°C for 5:00 minutes. The sample was held at 10°C. Each PCR cycle consisted of a denaturation step of 94°C for 1:00 minute, an annealing step for 1:00 minute at the annealing temperature indicated in table 2, and an extension step of 70°C for 2:30 minutes.

### **Gel Electrophoresis:**

Ten  $\mu$ l of PCR product was subjected to electrophoresis on an agarose gel (0.8% agarose, 100 mM TBE) at 120 volts for 45 minutes. The gel was stained with ethidium bromide for 10 min and destained with ddH<sub>2</sub>O for 20 min. The bands were visualized under UV-light.

### **PCR Purification:**

The PCR products were purified using the GENECLAN Turbo for PCR Kit and the GENECLAN III Kit (both from Q.Biogene, Inc. Carlsbad, CA) according to the manufacturer's directions, except ddH<sub>2</sub>O was used to elute the DNA instead of the elution buffer enclosed in the kit.

### **Cloning and Transforming Cells:**

Purified PCR product was ligated into the plasmid vector, pCR4-TOPO, using the TOPO TA cloning kit from Invitrogen (Carlsbad, CA). The plasmid was used to transform Top 10 cells according to the manufacturer's directions. Cells were plated on LB-Amp plates and incubated overnight at 37°C.

### **Plasmid Mini Preps**

Transformed bacteria growing on LB-Amp plates were used to inoculate 5 ml LB-Amp cultures. Cultures were incubated at 37°C overnight with shaking. The next day, the tubes were centrifuged, the cells were pelleted and the medium was aspirated. Plasmids were harvested from cells using Qiagen plasmid miniprep kit (Valencia, CA) as described in the manufacturer's manual. In the final step, ddH<sub>2</sub>O was used to elute plasmid DNA instead of elution buffer.

### **Restriction Digest:**

Plasmid DNA was digested with EcoR I (Promega, Madison, WI). The reaction was held at 37°C for an hour.

### **DNA Sequencing:**

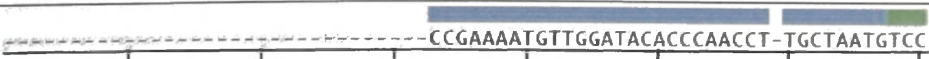
The 730 bp, 720 bp, 550 bp and 540 bp fragments of ND2 and tRNA<sup>trp</sup> were sent directly for DNA sequencing without cloning into plasmid. The 1200 bp fragment was cloned into PCR4-TOPO plasmid vector prior to DNA sequencing. All samples were sent to the DNA Sequencing Core at the University of Michigan – Ann Arbor. The sequences were viewed and analyzed using Lasergene 6.1 (DNA-STAR, Madison, WI).

**Sequence Analysis:**


The sequences were viewed and analyzed using Lasergene 6.1 (DNA-STAR, Madison, WI). Sequences were aligned using Clustal W method (DNA-STAR, Madison, WI). Phylogenetic trees were constructed with maximum parsimony using PAUP 4.0 (Sinauer Associates, Inc. Sunderland, MA).

## **Results:**

The ND2 and tRNA<sup>trp</sup> amplified sequences were obtained from 20 lizard tissue samples. Seven samples were sequenced from Michigan, two from Indiana, three from Arkansas, three from Texas, three from Florida and two from Nebraska. All were *Aspidoscelis sexlineata viridis* except the three Florida samples, which were *Aspidoscelis sexlineata sexlineata*. The DNA sequences were compared to each other and to the published ND2 sequence of *Ameiva chrysolema* (Accession Number: AY561703). *Ameiva chrysolema* is used as an outgroup taxon. The data set includes 1227 aligned sites from 20 ingroup taxa (Figure 6). *Aspidoscelis* and *Ameiva* both belong to the Teiid family. The aligned sequences show some differences that were analyzed by constructing a phylogenetic tree using maximum parsimony. The constructed tree is rooted. Of the aligned 1227 nucleotide sites, 65 are parsimony-informative.


  
 10 20 30 40 50 60 70

Ameiva c. -----TGTC 5  
 A.s.v.AR1 -----CCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 1127  
 A.s.v.AR2 -----CCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 38  
 A.s.v.AR3 ----- 1  
 A.s.v.IN1 ----- 1  
 A.s.v.IN2 -----TCAGGTTAAGCTTTTCGGGCCCATACCCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 64  
 A.s.v.MI1 -----CCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 38  
 A.s.v.MI2 -----CCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 1127  
 A.s.v.MI3 -----CCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 38  
 A.s.v.MI4 -----GCTTTTCGGGCCCATACCCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 1145  
 A.s.v.MI5 -----TAGCTTTTCGGGCCCATACCCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 57  
 A.s.v.MI6 -----GCTTTTCGGGCCCATACCCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 55  
 A.s.v.MI7 ----- 1  
 A.s.s.FL1 GGTACGATTGCTTAGCTTTTCGGGCCCATACCCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 70  
 A.s.s.FL2 ----- 1078  
 A.s.s.FL3 ----- 513  
 A.s.v.NE1 -----TCC 1093  
 A.s.v.NE2 -----CCGAAAATGTTGGATACACCCAACCTTTGCTAATGTCC 1100  
 A.s.v.TX1 ----- 1076  
 A.s.v.TX2 ----- 1061  
 A.s.v.TX3 ----- 1056


  
 80 90 100 110 120 130 140

Ameiva c. CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 74  
 A.s.v.AR1 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 1058  
 A.s.v.AR2 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 107  
 A.s.v.AR3 ----- 1  
 A.s.v.IN1 -----CCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 68  
 A.s.v.IN2 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 133  
 A.s.v.MI1 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 107  
 A.s.v.MI2 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 1058  
 A.s.v.MI3 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 107  
 A.s.v.MI4 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 1076  
 A.s.v.MI5 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 126  
 A.s.v.MI6 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 124  
 A.s.v.MI7 -----TAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 65  
 A.s.s.FL1 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 139  
 A.s.s.FL2 -----CTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 1040  
 A.s.s.FL3 ----- 513  
 A.s.v.NE1 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 1023  
 A.s.v.NE2 CCCCTAATCAACAG-CATTTTAATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 1031  
 A.s.v.TX1 -----CATTTTATTCTATCCCTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 1023  
 A.s.v.TX2 -----CTAATCACCAGAACCTTAATCACAATAAACAGTAACAC 1023  
 A.s.v.TX3 -----CACCAGAACCTTAATCACAATAAACAGTAACAC 1023

- - - - - + - : less similar + : more similar





CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT  
 290 300 310 320 330 340 350

Ameiva c. CTCAAGCTGCATTAAAGCCTGACACTCAGGAACATGAGATATTACTCAACTAACGAACCCCTAGCCTGT 284  
 A.s.v.AR1 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 848  
 A.s.v.AR2 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 317  
 A.s.v.AR3 ----- 1  
 A.s.v.IN1 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 278  
 A.s.v.IN2 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 343  
 A.s.v.MI1 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 317  
 A.s.v.MI2 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 848  
 A.s.v.MI3 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 317  
 A.s.v.MI4 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 866  
 A.s.v.MI5 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 336  
 A.s.v.MI6 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 334  
 A.s.v.MI7 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 275  
 A.s.s.FL1 CGCAAGCTCCACTAAAGCCTGGCAGACAGGAACATGAGACATTACCCCAACTTATAAACCCCATATCATGT 349  
 A.s.s.FL2 CGCAAGCTCCACTAAAGCCTGGCAGACAGGAACATGAGACATTACCCCAACTTATAAACCCCATATCATGT 830  
 A.s.s.FL3 ----- 513  
 A.s.v.NE1 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 813  
 A.s.v.NE2 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 821  
 A.s.v.TX1 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 813  
 A.s.v.TX2 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 813  
 A.s.v.TX3 CGCAAGCTCCACTAATGCCTGACACACAGGAACATGAGATATTACTCAACTTATAAACCCCATATCATGT 813

ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTGTGACTGCCAGAAGTAA  
 360 370 380 390 400 410 420

Ameiva c. ACCACACTAATATTGCGCTGTGCATAAAAATGGACTACCCCAATACACCTGTGACTACCAGAAGTAA 354  
 A.s.v.AR1 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTATGACTGCCAGAAGTAA 778  
 A.s.v.AR2 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTATGACTGCCAGAAGTAA 387  
 A.s.v.AR3 ----- 1  
 A.s.v.IN1 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTGTGACTGCCAGAAGTAA 348  
 A.s.v.IN2 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTGTGACTGCCAGAAGTAA 413  
 A.s.v.MI1 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTGTGACTGCCAGAAGTAA 387  
 A.s.v.MI2 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTGTGACTGCCAGAAGTAA 778  
 A.s.v.MI3 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTATGACTGCCAGAAGTAA 387  
 A.s.v.MI4 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTGTGACTGCCAGAAGTAA 796  
 A.s.v.MI5 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTATGACTGCCAGAAGTAA 406  
 A.s.v.MI6 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTATGACTGCCAGAAGTAA 404  
 A.s.v.MI7 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTGTGACTGCCAGAAGTAA 345  
 A.s.s.FL1 ACAGCACTCACCATTGCCCTCTGTATAAAAAATGGCTTAGCCCCAATGCACCTATGACTACCAGAAGTAA 419  
 A.s.s.FL2 ACAGCACTCACCATTGCCCTCTGTATAAAAAATGGCTTAGCCCCAATGCACCTATGACTACCAGAAGTAA 760  
 A.s.s.FL3 ----- 513  
 A.s.v.NE1 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTATGACTGCCAGAAGTAA 743  
 A.s.v.NE2 ACAGCACTCACCATTGCCCTATGTATAAAAAATGGATTAGCCCCAATGCACCTATGACTGCCAGAAGTAA 751  
 A.s.v.TX1 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTGTGACTGCCAGAAGTAA 743  
 A.s.v.TX2 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTGTGACTGCCAGAAGTAA 743  
 A.s.v.TX3 ACAGCACTCACCATTGCCCTATGTATAAAAAATCGGATTAGCCCCAATGCACCTGTGACTGCCAGAAGTAA 743

— + -: less similar +: more similar

		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	
	430	440	450
Ameiva c.		TACAAGGAACCACTAAAAAGAGCCCTCATTATTGCAACCTGACAAAAATTAGCTCCAATAAGCTTAT	424
A.s.v. AR1		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	708
A.s.v. AR2		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	457
A.s.v. AR3		1	
A.s.v. IN1		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	418
A.s.v. IN2		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	483
A.s.v. MI1		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	457
A.s.v. MI2		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	708
A.s.v. MI3		TACAAGGAACAACCTTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	457
A.s.v. MI4		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	726
A.s.v. MI5		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	476
A.s.v. MI6		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	474
A.s.v. MI7		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	415
A.s.s. FL1		TACARGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	489
A.s.s. FL2		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	690
A.s.s. FL3		513	
A.s.v. NE1		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	673
A.s.v. NE2		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	681
A.s.v. TX1		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	673
A.s.v. TX2		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	673
A.s.v. TX3		TACAAGGAACAACCTCTAAAAGTAGCTCTCATTATTGCAACATGACAAAAATTAGCTCCAATAAGTCTAAT	673

		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	
	500	510	520
Ameiva c.		CATCATAACAGCAACAGCCACTCTAAATTTCTACTAATAACAATAGCCACCCTTTCTATTTTAGTTGGG	494
A.s.v. AR1		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	638
A.s.v. AR2		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	527
A.s.v. AR3		1	
A.s.v. IN1		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	488
A.s.v. IN2		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	553
A.s.v. MI1		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	527
A.s.v. MI2		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	638
A.s.v. MI3		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	527
A.s.v. MI4		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTGTACTCATAGCAACATTATCTATTGTTATTGGA	656
A.s.v. MI5		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	546
A.s.v. MI6		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	544
A.s.v. MI7		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	485
A.s.s. FL1		CCTRATAACATCTAATAGTCATTCAAACTTCTACTTATTCTCATAGCAACACTATCCATTATCATTGGA	559
A.s.s. FL2		CCTAATAACATCTAATAGTCATTCAAACTTCTACTTATTCTCATAGCAACACTATCCATTATCATTGGA	620
A.s.s. FL3		513	
A.s.v. NE1		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	603
A.s.v. NE2		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	611
A.s.v. TX1		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	603
A.s.v. TX2		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	603
A.s.v. TX3		CCTAATAACATCCAATAGTCACTCAAACTTCTACTTATACTCATAGCAACATTATCTATTGTTATTGGA	603



 -: less similar    +: more similar




	570	580	590	600	610	620	630	
	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							
Ameiva c.	GGCTGAGGGGGGAATCAACCAAAACACAGCTACGAAAAATCATAGCCTACTCCTCTATCGCAACATAGGAT							564
A.s.v. AR1	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							568
A.s.v. AR2	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							597
A.s.v. AR3								1
A.s.v. IN1	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							558
A.s.v. IN2	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							623
A.s.v. MI1	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							597
A.s.v. MI2	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							568
A.s.v. MI3	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							597
A.s.v. MI4	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							586
A.s.v. MI5	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							616
A.s.v. MI6	GGATGAGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							614
A.s.v. MI7	GGATGAGGGGGGACTAAAYCAAACMAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							555
A.s.s. FL1	GGATGGGGGGGACTAAACCAAACACAACCTACGAAAAATCATGGCTTACTCTCCATCGCTAATATAGGAT							629
A.s.s. FL2	GGATGGGGGGGACTAAACCAAACACAACCTACGAAAAATCATGGCTTACTCTCCATCGCTAATATAGGAT							550
A.s.s. FL3								513
A.s.v. NE1	GGATGAGGGGGGACTAAACCAAACCAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							533
A.s.v. NE2	GGATGGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							541
A.s.v. TX1	GGATGGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							533
A.s.v. TX2	GGATGGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							533
A.s.v. TX3	GGATGGGGGGGACTAAATCAAACACAACCTACGAAAAATCATGGCCTATTCTCCATCGCTAATATAGGAT							533

	640	650	660	670	680	690	700	
	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							
Ameiva c.	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							634
A.s.v. AR1	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTTCTATATATCATAAGTAAC							498
A.s.v. AR2	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTTCTATATATCATAATAAC							667
A.s.v. AR3	-----CAAGCACCACCAAACTCTCAACACTTACTCTTCTTCTATATATCATAATAAC							50
A.s.v. IN1	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							628
A.s.v. IN2	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							693
A.s.v. MI1	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							667
A.s.v. MI2	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							498
A.s.v. MI3	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							667
A.s.v. MI4	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							516
A.s.v. MI5	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							686
A.s.v. MI6	GAATAATTATTATTTTACAACMAGCACCACCAAACTCTCAACACTYACTCTTCTCCTATATATCATAATAAC							684
A.s.v. MI7	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							625
A.s.s. FL1	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACCCTCCTCCTATACATCATAATAAC							699
A.s.s. FL2	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACCCTCCTCCTATACATCATAATAAC							480
A.s.s. FL3	-----AACACTTACCCTCCTCCTATACATCATAATAAC							481
A.s.v. NE1	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							463
A.s.v. NE2	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							471
A.s.v. TX1	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							463
A.s.v. TX2	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							463
A.s.v. TX3	GAATAATTATTATTTTACAACAAGCACCACCAAACTCTCAACACTTACTCTTCTCCTATATATCATAATAAC							463

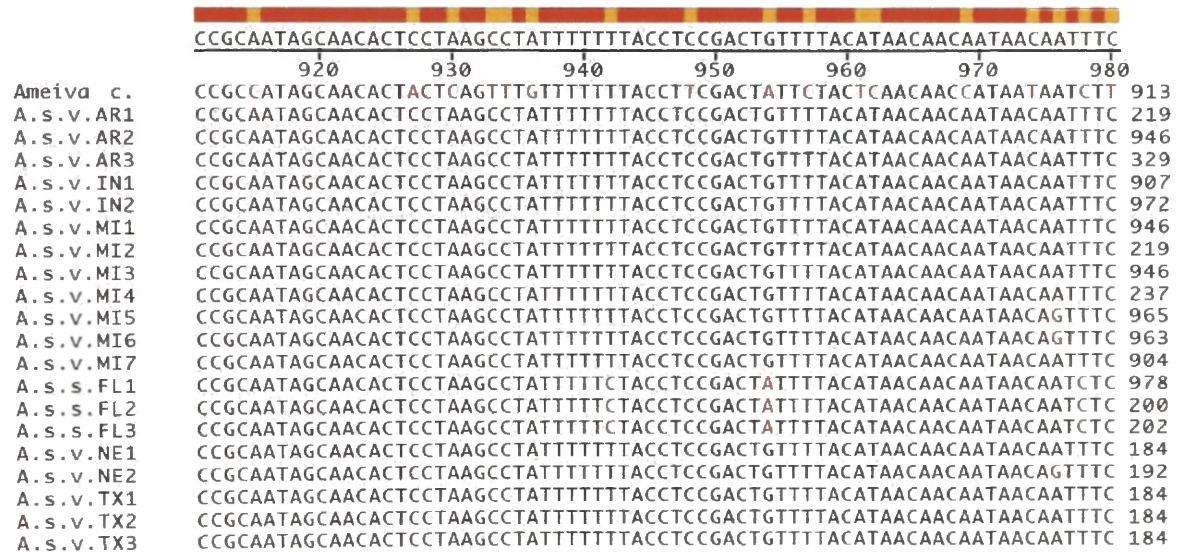

 -: less similar    +: more similar

		
	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	
	710 720 730 740 750 760 770	
Ameiva c.	CACAACAATATTTATAACACTAATACCACAAAAACAAAAACATCAAAACAATTGGTACAACATGAAT-	704
A.s.v. AR1	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	428
A.s.v. AR2	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	737
A.s.v. AR3	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	120
A.s.v. IN1	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	698
A.s.v. IN2	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	763
A.s.v. MI1	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	737
A.s.v. MI2	CACAACGATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	428
A.s.v. MI3	CACAACAATATTCATAGCATTAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	737
A.s.v. MI4	CGCAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	446
A.s.v. MI5	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	756
A.s.v. MI6	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	754
A.s.v. MI7	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	695
A.s.s. FL1	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	769
A.s.s. FL2	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	410
A.s.s. FL3	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	411
A.s.v. NE1	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	393
A.s.v. NE2	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	401
A.s.v. TX1	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	393
A.s.v. TX2	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	393
A.s.v. TX3	CACAACAATATTCATGGCAATAACACCCCAAAAAACAAAAACAATTAAAAATAATCGGAACAACATGAAC-	393

		
	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	
	780 790 800 810 820 830 840	
Ameiva c.	ACTATCACCGTCCCTAACCACTATCATAATACTAACACTAACCTCTCTCGGAGGTCTCCACCTATAACA	773
A.s.v. AR1	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	359
A.s.v. AR2	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	806
A.s.v. AR3	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	189
A.s.v. IN1	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	767
A.s.v. IN2	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	832
A.s.v. MI1	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	806
A.s.v. MI2	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	359
A.s.v. MI3	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	806
A.s.v. MI4	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	377
A.s.v. MI5	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	825
A.s.v. MI6	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	823
A.s.v. MI7	ACTATCACCCCACTTACAATTATTATAATACTCACACTAACATCCCTAGGAGGACTCCCAACATAACA	764
A.s.s. FL1	ACTATCACCCCACTTACARTTATTATAATACTTACACTAACATCCCTAGGAGGACTCCCAACATAACA	838
A.s.s. FL2	ACTATCACCCCACTTACAATTATTATAATACTTACACTAACATCCCTAGGAGGACTCCCAACATAACA	340
A.s.s. FL3	ACTATCACCCCACTTACAATTATTATAATACTTACACTAACATCCCTAGGAGGACTCCCAACATAACA	342
A.s.v. NE1	ACTATCACCCCACTTACAATTATTATAATACTTACACTAACATCCCTAGGAGGACTCCCAACATAACA	324
A.s.v. NE2	ACTATCACCCCACTTACAATTATTATAATACTTACACTAACATCCCTAGGAGGACTCCCAACATAACA	332
A.s.v. TX1	ACTATCACCCCACTTACAATTATTATAATACTTACACTAACATCCCTAGGAGGACTCCCAACATAACA	324
A.s.v. TX2	ACTATCACCCCACTTACAATTATTATAATACTTACACTAACATCCCTAGGAGGACTCCCAACATAACA	324
A.s.v. TX3	ACTATCACCCCACTTACAATTATTATAATACTTACACTAACATCCCTAGGAGGACTCCCAACATAACA	324


 -: less similar    +: more similar






29

		990	1000	1010	1020	1030	1040	1050	
		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							
Ameiva c.		CCCCAACACAACAGCTACACAAAAATGGCGACATCAAATAAAAAACCATAACCATCATAATACTAAC							983
A.s.v. AR1		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							149
A.s.v. AR2		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							1016
A.s.v. AR3		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							399
A.s.v. IN1		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							977
A.s.v. IN2		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							1042
A.s.v. MI1		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							1016
A.s.v. MI2		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							149
A.s.v. MI3		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTGACT							1016
A.s.v. MI4		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							167
A.s.v. MI5		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							1035
A.s.v. MI6		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							1033
A.s.v. MI7		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							974
A.s.s. FL1		CCCCAATACAATATATACGACACAAAAATGACGATTACAAACAAAAACCATAACAATTATTATATTGACT							1048
A.s.s. FL2		CCCCAATACAATATATACGACACAAAAATGACGATTACAAACAAAAACCATAACAATTATTATATTGACT							130
A.s.s. FL3		CCCCAATACAATATATACGACACAAAAATGACGATTACAAACAAAAACCATAACAATTATTATATTGACT							132
A.s.v. NE1		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTATTATATTAAC							114
A.s.v. NE2		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTAAC							122
A.s.v. TX1		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTGACT							114
A.s.v. TX2		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTGACT							114
A.s.v. TX3		CCCCAACACAATAAACACAACACAAAAATGACGATTACAAACAAAAACCATAATAATTGTTATATTGACT							114

		1060	1070	1080	1090	1100	1110	1120	
		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							
Ameiva c.		ACGCCCCGAGCACTAATACTACTCCCCAATCCTCCCAATCACACCCCATAGAAATATAGGCTAATCATT							1053
A.s.v. AR1		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							79
A.s.v. AR2		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							1086
A.s.v. AR3		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							469
A.s.v. IN1		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							1047
A.s.v. IN2		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							1112
A.s.v. MI1		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							1086
A.s.v. MI2		TCCCCGGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							79
A.s.v. MI3		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							1086
A.s.v. MI4		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							97
A.s.v. MI5		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							1105
A.s.v. MI6		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							1103
A.s.v. MI7		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							1044
A.s.s. FL1		TCCCCAGCAACTCTAATACTTCTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATATATT							1118
A.s.s. FL2		TCCCCAGCAACTCTAATACTTCTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATATATT							60
A.s.s. FL3		TCCCCAGCAACTCTAATACTTCTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATATATT							62
A.s.v. NE1		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							44
A.s.v. NE2		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							52
A.s.v. TX1		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							44
A.s.v. TX2		TCCCCAGCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							44
A.s.v. TX3		TCCCCATCAACTCTAATACTACTCCCCATTCTTCCCCTAATTAATCCATAAGAAATATAGGCTATCTATT							44


 -: less similar    +: more similar



**Figure 6: Alignment of ND2 and tRNA<sup>trp</sup> sequences of *Aspidoscelis sexlineata sexlineata* (A.s.s), *Aspidoscelis sexlineata viridis* (A.s.v) and *Ameiva chrysolaema* (Ameiva c.).** Approximately 1227 bp of sequence was compared. For samples AR3 and FL3 only 550 bp of sequence was obtained. Nucleotides that match the consensus sequence are shown in black. Nucleotides that differ from the consensus sequence are shown in red. Nucleotides that are present in some taxa but are absent in others are shown in blue. MI: Michigan. IN: Indiana. AR: Arkansas. NE: Nebraska. TX: Texas. FL: Florida.



Molecular phylogenetic methods often produce several equally parsimonious trees; however, it is typically difficult to present all of the trees. One way to solve this problem is to make a composite tree that represents all of the trees, and such a tree is called a consensus tree. One type of consensus tree is the majority rule consensus tree. A 50%-majority rule consensus tree adopts a branching pattern that occurs with a frequency of 50% or more in a number of equally parsimonious trees. The higher the majority rule percent the less branching is likely to appear in the consensus tree. A 50%-majority rule consensus tree is mostly commonly used. Posterior probability is indicated on the branches of the 50%-majority rule consensus tree. Posterior probability is the conditional probability that is assigned after the scientific evidence (nucleotide differences) is taken into account. Conditional probability is the probability of event A (production of clade A) given the occurrence of event B (production of clade B).

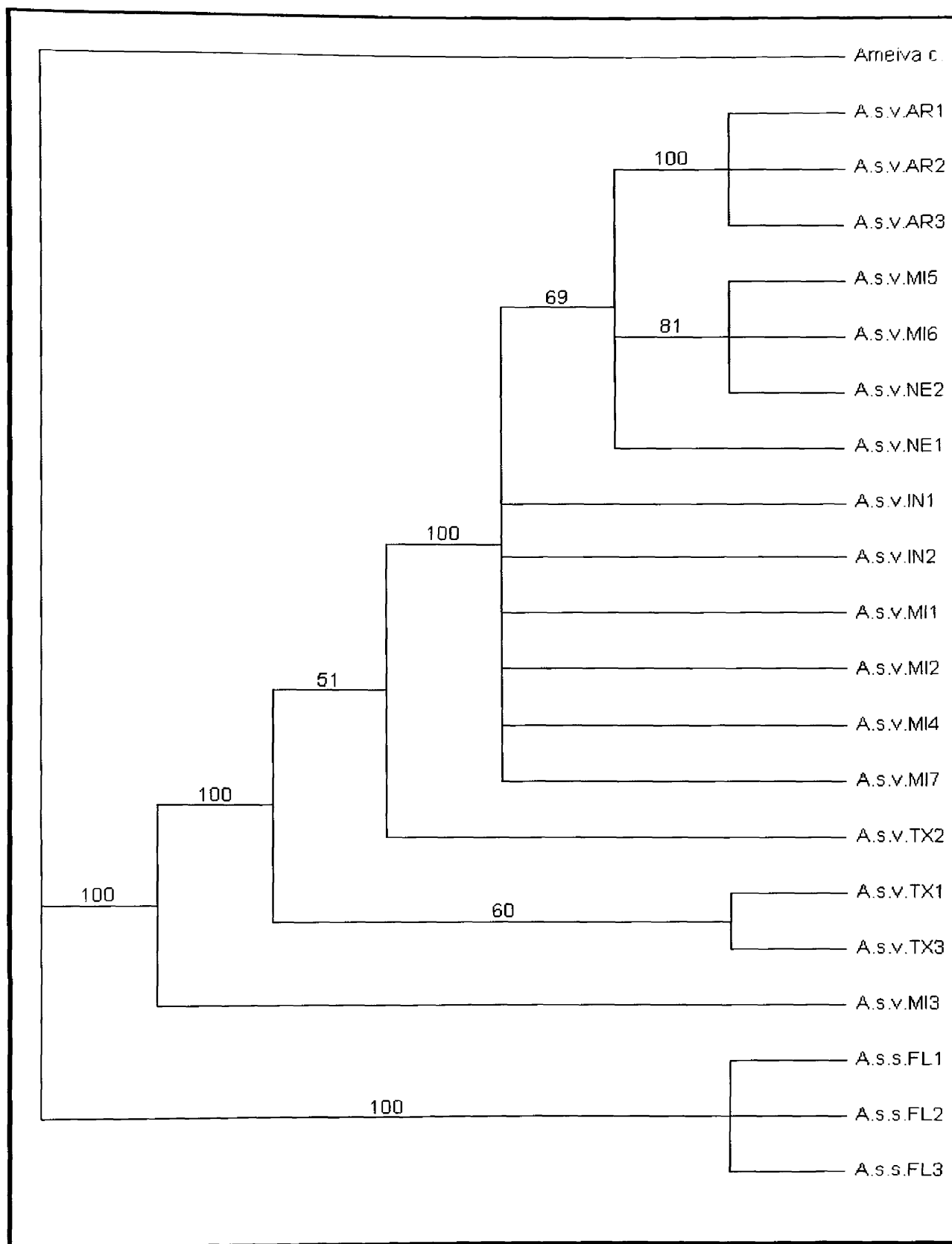
One effective way of testing the reliability of a molecular phylogenetic tree is to use the bootstrap test. A set of nucleotide sites is randomly sampled with replacement from the original set. This random set that has the same number of nucleotide



sites as that of the original set is used for constructing a new tree. The reliability of the inferred tree is evaluated by the percentage of times in which each branching pattern is found among all replicate bootstrap trees (Nei and Kumar, 2000). A consensus tree is constructed from the replicate bootstrap trees. This procedure has the advantage of avoiding multifurcating trees by producing a low-percent majority-rule consensus tree. A bootstrap value with reasonable support for a clade is roughly 70% and with high support is 95% (Felsenstein, 1985). A 50%-majority rule consensus tree and a Bootstrap consensus tree produce phylogenetic trees depending on the frequency of the branching pattern yet they sample the sequences using different techniques.

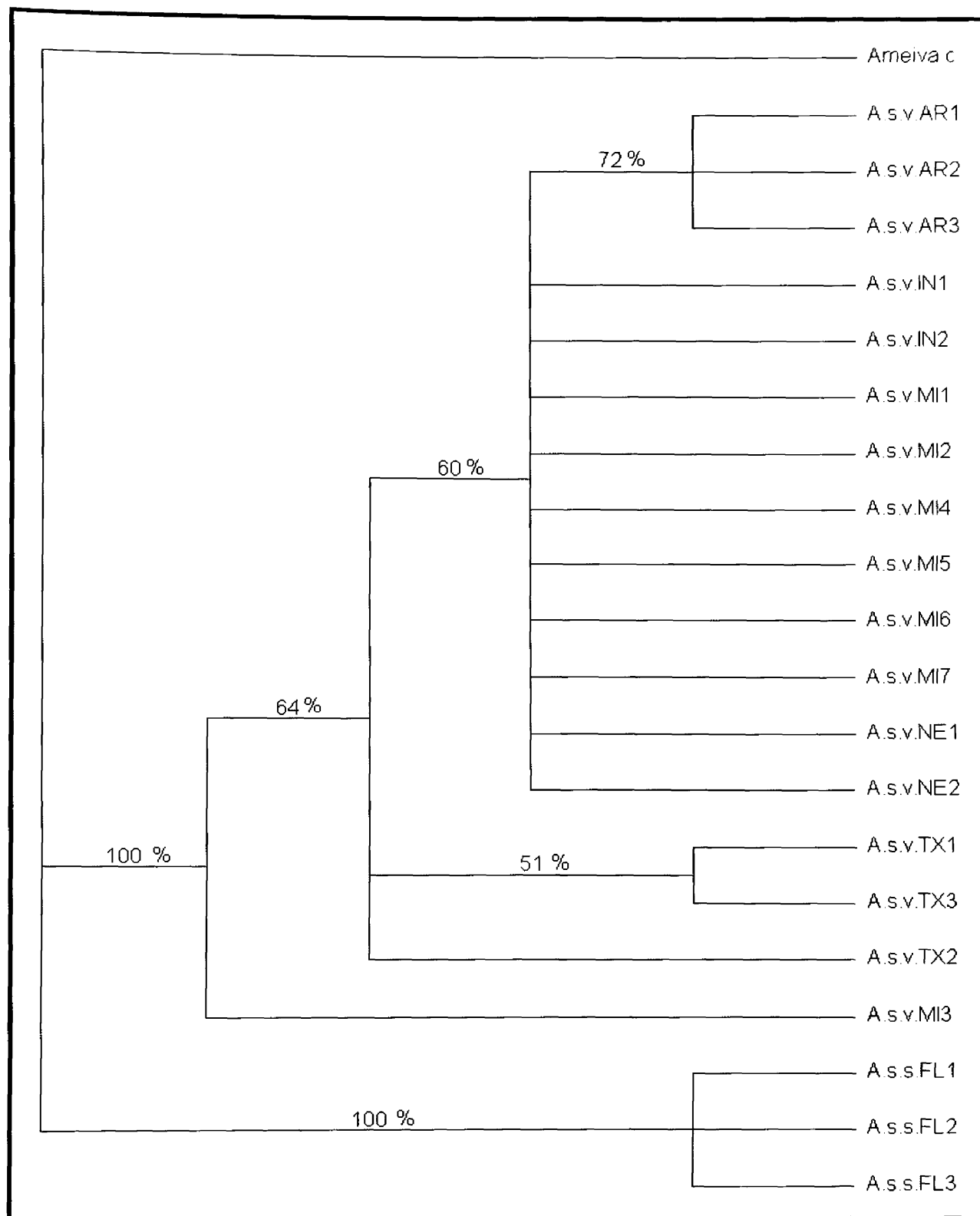
A 50%-majority rule consensus tree (Figure 7) and a bootstrap consensus tree based on maximum parsimony (Figure 8) were produced. Both the 50% majority-rule and bootstrap consensus trees are congruent. In the 50% majority-rule consensus tree, Florida samples form their own clade (posterior probability 100%). One Michigan sample, A.s.v. MI3 diverges early from the rest of the Midwestern samples (posterior probability 100%). This clade appears to be more related to Florida and Texas

samples than it is to the Midwestern and Arkansas samples. Texas samples are grouped together (posterior probability 100%), so that A.s.v TX1 and A.s.v TX3 are more closely related than A.s.v TX2 (posterior probability 51%). Nebraska, Indiana and most Michigan samples are all in the same cluster as expected, yet they are further divided into three groups. One clade consists of Indiana samples (A.s.v IN1, A.s.v IN2) and four of the Michigan samples (A.s.v MI1, A.s.v MI2, A.s.v MI4 and A.s.v MI7) (posterior probability 69%). The other clade consists of the other two Michigan samples (A.s.v MI5 and A.s.v MI6) and one Nebraska sample (A.s.v NE2), while the other Nebraska sample (A.s.v NE1) is in a third clade (posterior probability 81%). Arkansas samples resolve to produce a separate clade (posterior probability 100%). A.s.v MI5, A.s.v MI6, A.s.v NE1 and A.s.v NE2 appear to be more closely related to the Arkansas clade than to the other Midwestern samples.



**Figure 7: 50% Majority-rule Consensus Tree of 20 *Aspidoscelis sexlineata* Samples.** Florida and Texas samples diverge to produce two clades early in the tree. The Midwestern samples cluster with Arkansas samples, yet A.s.v MI5, MI6, NE1 and NE2 are more closely related to Arkansas samples than the other Midwestern ones. One Michigan sample is more closely related to Texas and Florida samples than to the Midwestern samples. This tree was generated based on maximum parsimony. A.s.s: *Aspidoscelis sexlineata sexlineata*, A.s.v: *Aspidoscelis sexlineata viridis* and Ameiva c: *Ameiva chrysolema*. MI: Michigan. IN: Indiana. AR: Arkansas. NE: Nebraska. TX: Texas. FL: Florida.

In the bootstrap consensus tree, Florida samples diverge the earliest from all *Aspidoscelis sexlineata viridis* (bootstrap 100%). A.s.v MI3 again resolves from all previous clades into a separate clade of its own (bootstrap 64%). The Texas samples are resolved from Arkansas, Indiana and Michigan taxa into a clade (bootstrap 60%). Yet, two of the Texas samples, A.s.v TX1 and A.s.v TX3, produce a subgroup within the Texas cluster. (bootstrap 51%). The clade containing Arkansas, Indiana, Nebraska and Michigan taxa diverges to produce two subgroups; One subgroup contains Arkansas samples and the other contains most of the Midwestern samples, including all Indiana, Nebraska and Michigan samples except A.s.v. MI3 (bootstrap 72%).



**Figure 8: Bootstrap Consensus Tree from 20 *Aspidoscelis sexlineata* Samples.** The Midwestern samples are more closely related to each other than to the western or southern taxa. One Michigan sample is more closely related to Texas and Florida samples than to the Midwestern samples. A.s.s: *Aspidoscelis sexlineata sexlineata*, A.s.v: *Aspidoscelis sexlineata viridis* and Ameiva c: *Ameiva chrysolema*. MI: Michigan. IN: Indiana. AR: Arkansas. NE: Nebraska. TX: Texas. FL: Florida.

## **Discussion:**

The results of this study supports the hypothesis that the Michigan population of Six-lined Racerunner is the subspecies *Aspidoscelis sexlineata viridis*, and this corroborates the morphological results and similar conclusion of Yoder (2007). So this population, whether endemic or introduced, is from the prairie *Aspidoscelis sexlineata viridis* populations in the Midwest or southwest US.

The two phylogenetic trees constructed from mitochondrial DNA analysis elucidate how populations of *Aspidoscelis sexlineata* from Arkansas, Nebraska, Michigan, Indiana, Texas and Florida are related. Both types of phylogenetic trees show similar grouping of samples; Arkansas clade, Midwestern clade, Texas clade and Florida clade. In the 50% majority-rule consensus tree, the Midwestern clade is further subdivided so that some Michigan samples (A.s.v MI1, A.s.v MI2, A.s.v MI4 and A.s.v MI7) and Indiana samples (A.s.v IN1 and A.s.v IN2) form one group and other Michigan samples (A.s.v MI5, A.s.v MI6) and Nebraska samples (A.s.v NE1 and A.s.v NE2) form another group, this second group is more closely related to the Arkansas clade than the other Midwestern samples. Texas

samples, being the farthest west, are not as closely related to the Michigan sequences as the Midwestern ones. The Florida samples are the most distantly related to Michigan sequences, which is expected, since the Florida population is of a different subspecies (*Aspidoscelis sexlineata sexlineata*). A.s.v MI3 is the only Michigan sample that did not resolve with the Midwestern clade; instead it formed a clade of its own which is more closely related to the Texas and Florida clades.

The Arkansas, Indiana, Nebraska, Michigan and Texas clades consisted of samples from the *Aspidoscelis sexlineata* subspecies *viridis*. The Florida clade has *Aspidoscelis sexlineata* samples that belong to the subspecies *A.s.sexlineata*, which is both morphologically and molecularly distinct from *A.s.viridis*.

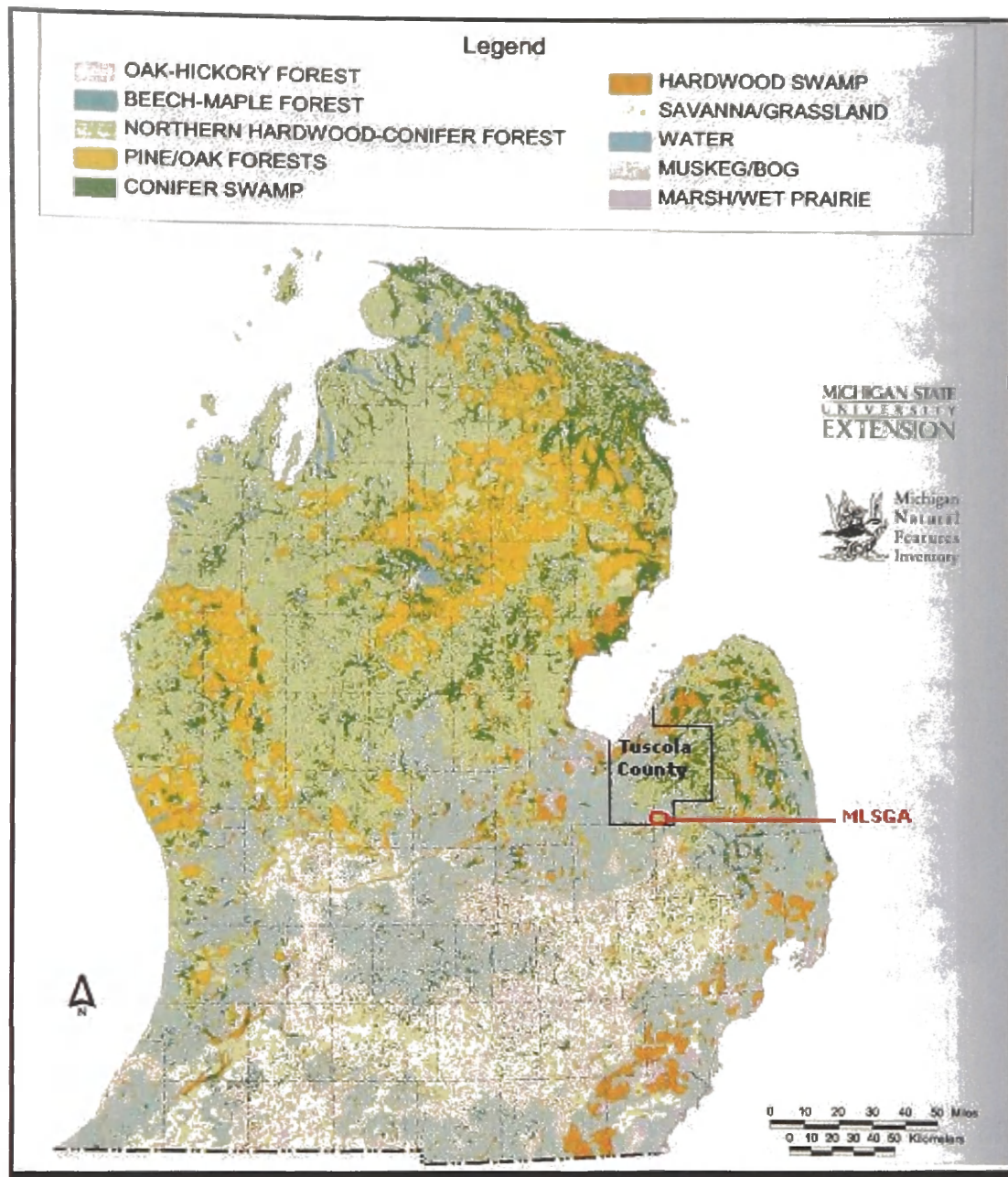
My hypothesis is that the *Aspidoscelis sexlineata* population in Michigan dispersed from a Midwestern population through the prairie habitat that extended from Indiana dunes to southern Michigan before European settlement. Due to urbanization, agriculture and road building, the Michigan population became isolated from the rest of the Midwest populations. The resulting phylogenetic trees support my hypothesis. A Midwestern clade



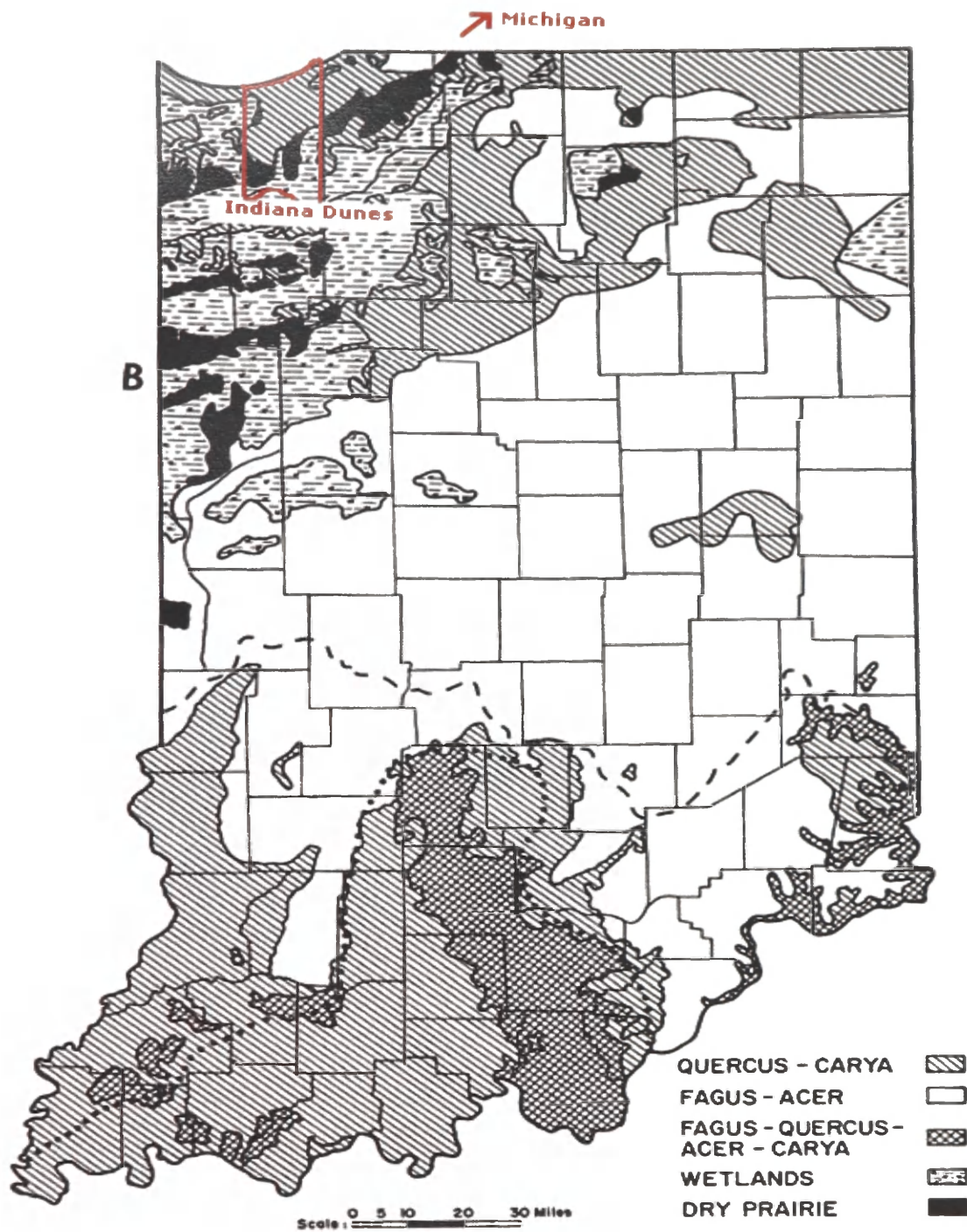
forms with samples from Michigan, Indiana and Nebraska. Another clade has the Arkansas samples. Texas and Florida samples each form their own clade early in the tree. One Michigan sample A.s.v MI3 has a similar sequence to Texas and Florida taxa. The data suggest that it is of the subspecies *A.s.viridis* because it did not resolve with the Florida clade. Yet, the overlapping zone of the two subspecies is not very far from the Midwestern populations that Michigan population might have dispersed from. A different gene sequence may be present in the population with low frequency due to possible interactions between the two subspecies in the overlapping zone. A larger sample size will give a better picture of this assumption.

The geographically closest *Aspidoscelis sexlineata* population to that in Michigan is found in the Indiana Dunes Region, over 322 km away (Conant & Collins, 1998; Harding, 1997). *Aspidoscelis sexlineata* distribution map shows the *A.s.viridis* range extending diagonally from the Southwestern states to northeastern Illinois, and ends up in northwestern Indiana. This narrow tongue might have extended into Michigan before European settlement.

Six-lined racerunners live in relatively dry regions on sandy or other loose soil, in short grass, sparse woods, or areas with scattered, subxerophytic vegetation. Dryness seems more essential than any other factor (Mcfarlane, 1999). The pre-settlement maps suggest that the dry prairie habitat in northwestern Indiana, where *A. sexlineata* is found today, extended into southern Michigan before European settlement (Figure 8; Figure 9). Although Tuscola County lies on the extreme northeastern edge of the savanna habitat in pre-settlement times, the Murphy Lake State Game Area is located in southern Tuscola County where the savanna habitat probably existed.



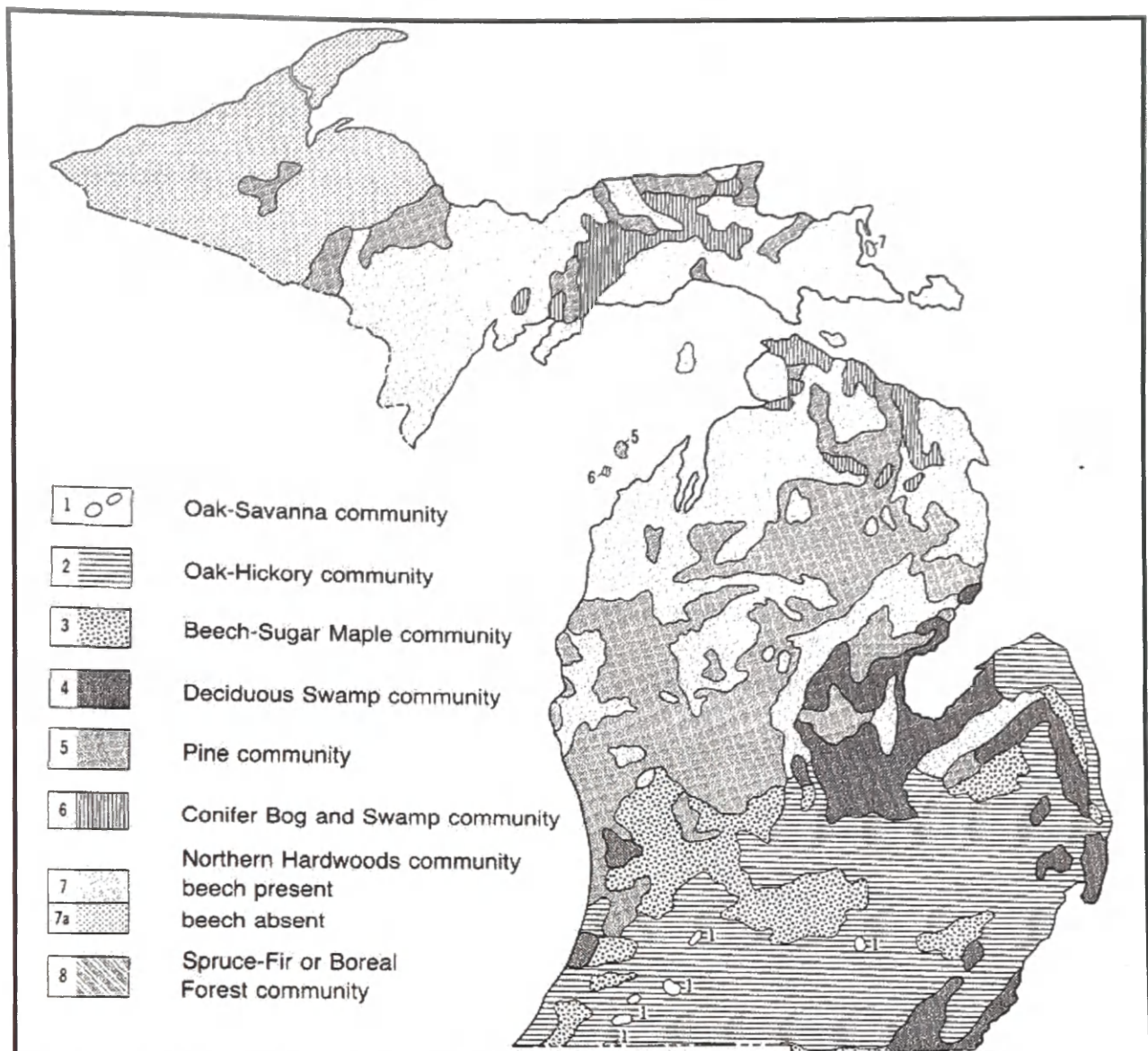
**Figure 9: Map of Pre-settlement Vegetation of Michigan, circa 1800 (Barnes & Wagner, 2004).** The savanna/grassland community (in yellow) extended from the southwestern corner of the state (nearest to the Indiana Dunes Region) northeast to Tuscola County. The MLSGA is indicated in Tuscola County.



**Figure 10: Map of Pre-settlement Vegetation of Indiana, circa 1820 (Lindsey, 1965).** Indiana Dunes are located in the prairie habitat in Indiana which extends into southern Michigan.

The current vegetation map of Michigan (Figure 11) shows an oak-hickory community in southern Michigan. The map shows a continuous area of oak-hickory forest, yet it is a mosaic of different communities that are simply too small to be shown on the map. Small remnants of oak-savanna are dispersed in the oak-hickory community although they were a major community in the pre-settlement vegetation of southern Michigan (Barnes & Wagner, 2004). The savanna habitat is found as dispersed patches in MLSGA. The area where the Six-lined Racerunners are found is a prairie/savanna community.





**Figure 11: Map of current vegetation in Michigan, circa 2004.** The oak-savanna community (region 1) has been reduced and is dispersed in an oak-hickory community in southern Michigan compared to that shown in the pre-settlement map of Michigan (Barnes & Wagner, 2004).

Murphy Lake State Game Area (MLSGA) is a collection of mostly forest communities in southern Tuscola County. It is a grouping of 'habitat islands' in a 'sea' of agriculture (Molles 2005). This is nothing unusual given the fragmented landscape of southeast Michigan. The most recent glacial period in North America, that started 70,000 years ago, has reformed its topography. Rifts and end moraines are left behind when glaciers receded. During that glacial period, some animals migrated south to warmer areas. After the glaciers receded, they migrated back north and inhabited the newly formed areas. Such animals represent glacial relicts. MLSGA's location along an end moraine interspersed with kettle ponds provides a testament to its relatively recent glacial history. What is unusual, however, is the presence of animal species that are found only in MLSGA and nowhere else in Michigan. Their distribution range is mainly in the southern and central areas. These animal species possibly represent 'glacial relicts'. It has been known for two decades that a disjointed population of six-lined racerunners, *Aspidoscelis sexlineata*, exists in MLSGA (Harding & Holman 1990). The nearest population of *Aspidoscelis sexlineata* is found in Indiana Dunes Region over 322 km away (Conant & Collins, 1998; Harding, 1997). *Aspidoscelis sexlineata* is found

in the southern to Midwestern parts of the United States (Figure 2). Recently a unique population of *Desmognathus fuscus* was also discovered in the area (Carlson, 2005).

Based on the physical appearance described by Yoder (2007) and molecular data provided by this study of *Aspidoscelis sexlineata viridis* in Michigan, the MLSGA population may be a glacial relict. The phylogenetic trees show that the Michigan samples are more closely related to the Midwestern samples from Indiana and Nebraska than to western samples from Texas or southern samples from Florida. Pre-settlement vegetation maps support the idea that the dry prairie habitat required by *Aspidoscelis sexlineata* once extended from Indiana dunes to MLSGA. The current vegetation map of Michigan shows remnants of the prairie habitat in the area. Even though some are too small to be shown on the map (Barnes & Wagner, 2004), MLSGA is one of those areas that has a dispersed patches of the prairie habitat. The fact that Six-lined Racerunners can forage for kilometers and their speed can go up to 29 km/h (Vogt. 1981), supports the idea that they naturally dispersed from Indiana Dunes to MLSGA.



Since the *Aspidoscelis sexlineata viridis* population in Michigan may represent a glacial relict, it is a priority to protect such an important relict and maintain an ancient species in Michigan. Racerunner populations in other parts of the Great Lakes area are localized and often isolated, and thus subject to rapid decline or extirpation if their open, sparsely vegetated habitats are modified or degraded (Harding 1997). Being adjacent to a busy road, the *Aspidoscelis sexlineata* habitat in MLSGA is exposed and vulnerable to destruction. Pesticides used on the road sides, vehicles parking in grassy areas, power lines and road maintenance can greatly affect the survival of these lizards. Management decisions should be made as soon as possible in order to protect this unique species in Michigan.

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